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Award Number: DAMD17-01-1-0433

TITLE: Molecular Tracking of Proteolysis during Breast Cancer Cell Extravasation: Blockage by Therapeutic Inhibitors

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REPORT DATE: August 2004

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2004	Final (1 Aug 2001 - 31 Jul 2004)

4. TITLE AND SUBTITLE
Molecular Tracking of Proteolysis during Breast Cancer Cell Extravasation: Blockage by Therapeutic Inhibitors

5. FUNDING NUMBERS
DAMD17-01-1-0433

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Elucidating the mechanism of focal proteolysis at the cell-matrix interface during metastasis is an ongoing challenge. We have completed the dissection of components of proteolytic machinery required for breast cancer cells (metastatic MDA-MB231 and non-metastatic MCF-7) transendothelial migration (TEM). Modulation of individual molecules demonstrates the functional cooperation of furin, cell surface adhesion molecules ($\alpha_v\beta_3$, CD44), and matrix metalloproteinases (MMP-2, MMP-9 and MT1-MMP) during the process of TEM. Confocal microscopy shows co-localization of molecules and MMPs critical for TEM, and disruption of these molecules reduces TEM of MDA-MB231 cells. MMP-2 and MT1-MMP localization matches the imprint of spatially restricted fluorogenic gelatin digestion. The digestion occurs in a stage-specific manner and becomes most evident during the migratory phase of tumor cell TEM. Of the above breast cancer cell lines, MDA-MB231 is able to undergo efficient TEM, and MT1-MMP emerges to be one of the key molecules involved for this event. The lack of several key components in MCF-7 cells impairs its ability to transmigrate. We have now extended our analyses of protease activity by these cells in vivo to the mammary gland, illustrating their differential capacity to degrade the matrix components in the transplanted plug. Our data reveal how specific molecular interactions result in a cooperative proteolytic interface at the metastatic tumor cell surface. We have shown this in vitro utilizing the breast tumor cell-endothelial cell microenvironment during TEM, and in vivo in the mammary tissue environment.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
7

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction:

Elucidating the degradative effectors responsible for focal proteolysis at the cell-matrix interface during metastasis is an ongoing challenge. We have completed the dissection of components of the proteolytic machinery required for breast cancer cell (metastatic MDA-MB231, and non-metastatic MCF-7) transendothelial migration (TEM). Specifically, we have determined the stage of extravasation that is particularly reliant upon metalloproteinase activity. In addition we tested the proteolytic capacity of these cells in vivo in the mouse mammary gland.

Body:

In the past year we had achieved the following: 1). Determined the MMP profile for the individual breast cancer cell lines MCF-7 and MDA-MB-231, and human lung microvascular endothelial cells by zymography. 2) Verified MT1-MMP and $\alpha v\beta 3$ expression at the protein level by western blotting. 3) Investigated the inhibitory effects of biological and synthetic MMP-inhibitors and a serine inhibitor. 4) Localized MMP-2, MMP-9, MT1-MMP and furin on the cancer cells during the process of TEM using antibodies. 5) Co-localized MMPs with cell surface molecules, including MMP-2 with $\alpha v\beta 3$, MT1-MMP with TIMP-2 during TEM, and while both CD44 and MMP-9 could be visualized on MDA-MB231 cells during TEM, their co-localization was not apparent. 6) Mapped proteolytic activity on individual cell as well as during the process of TEM and qualitatively assessed proteolytic-matrix degradation in situ at the tumor cell surface at distinct stages of TEM, by confocal microscopy.

Further to this, we have recently completed the following: 1) For specific manipulation of the individual complexes, we have utilized a) rCBD, b) HxCD (c-domain of MMP-2), c) $\alpha v\beta 3$ blocking peptide, d) an antibody against furin and rfurin, e) blocking antibodies against MT1-MMP or MMP-9. While HxCD, $\alpha v\beta 3$ blocking peptide and antibodies against furin, MT1-MMP and MMP-9 significantly decreased the process of TEM, rCBD and recombinant furin promoted this process. 2) Obtained MCF-7 cells transfected with either MT1-MMP, $\beta 3$ integrin subunit, or both from Dr. A. Strongin. These cells were tested for their ability to undergo TEM. These experiments yielded varying and inconclusive data with respect to their behaviour in the TEM assay.

To validate the differential TEM capacity of metastatic MDA-MB231 and non-metastatic MCF-7 cells, we transplanted cells in a Matrigel plug (containing fluorescent conjugated gelatin) in vivo in the mouse mammary gland and

visualized the proteolytic activity. MDA-MB231 cells showed far greater gelatin degradation compared to MCF-7 cells.

Key Research Accomplishments:

- Correlated TEM following the addition of rCBD which is known to induce MMP-2 activation (figure 1).
- Correlated inhibition of TEM following the addition of HxCD, the C-domain of MMP-2 (figure 2).
- Elucidated the mechanism of migration using blocking antibodies against MT1-MMP, MMP-9 and $\alpha v\beta 3$ (figures 3A-3C).
- Assessed changes in TEM following the addition of rfurin and furin blocking peptide (figure 4).
- Greater proteolytic digestion by MDA-MB231 cells compared to MCF-7 cells in vivo in the mouse mammary gland (figure 5).

Reportable Outcomes:

Two manuscripts are currently in preparation, which summarize these findings. The reprints from these will be forwarded for your records at a later date..

This data has been presented at the following meeting:

1. Protease Retreat. Wayne State University, Detroit, Michigan. May, 2004

Conclusions:

We can now conclude that the two breast cancer cell lines, one metastatic (MDA-MB231) and the other non-metastatic (MCF-7), significantly differ in their ability to undergo transendothelial migration. One basis for this differential TEM is that MCF-7 cells lack MT1-MMP as well as the $\beta 3$ integrin subunit. Through the use of blocking antibodies, we have confirmed the importance of these molecules and also of MMP-9 in TEM efficiency. Further, we have shown the importance of MMP-2 activity through the use of MMP-2 activity modifying reagents such as HxCD and rCBD. Specific transfections of MT1-MMP, $\beta 3$, or both showed inconclusive data in their ability to undergo TEM. .

Moreover, the differential proteolytic activity of the two breast cancer cell lines has been tested in vivo the mouse mammary gland, the orthotopic site for breast cancer cells. Again, we observe higher proteolytic activity and focused digestion with MDA-MB231 cells compared to MCF-7 cells.

The above progress has met and surpassed the milestones projected in the original application.

Key Research Accomplishments. Figures 1-4.

Fig.1

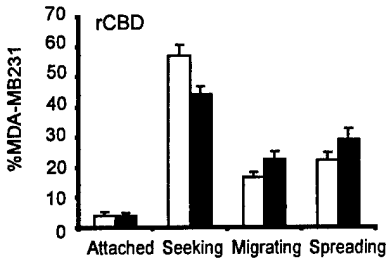


Fig. 2

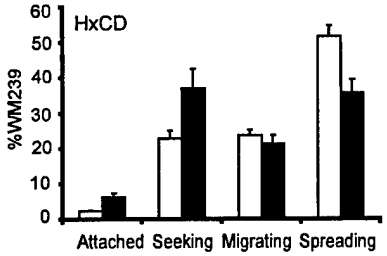


Fig. 3

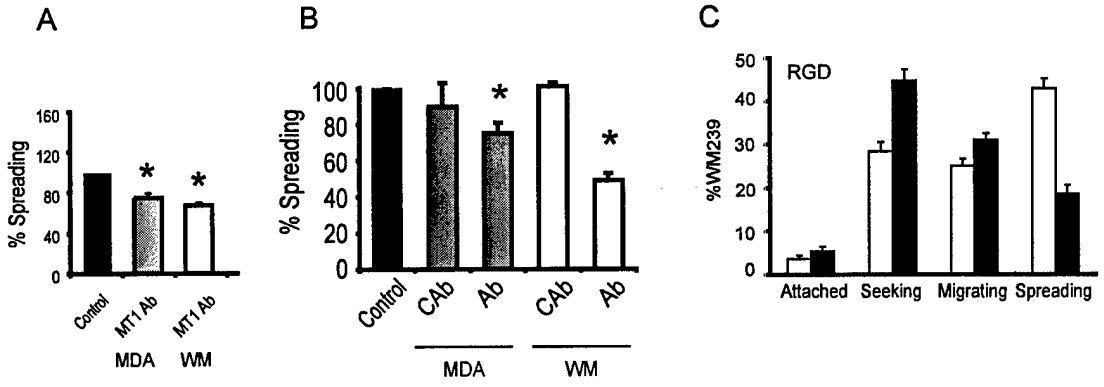
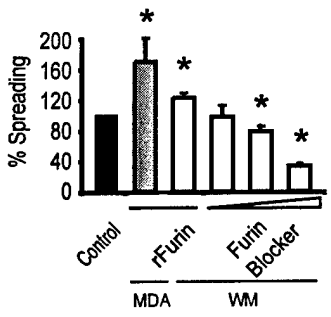


Fig. 4



Key Research Accomplishments. Figure 5.